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Interference of soil contaminants with laccase activity during the transformation of complex mixtures of polycyclic aromatic hydrocarbons in liquid media

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Introduction

Laccase-producing fungi are often presented as efficient tools for the bioremediation of soils and liquid wastes polluted by PAHs, but the efficiency of the enzymes has not been clearly demonstrated

Aims of the study:

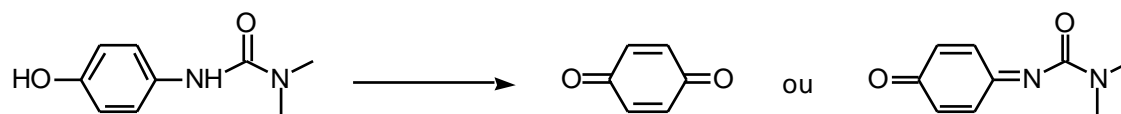
- to determine the efficiency of fungal laccases to transform synthetic mixtures of PAHs
- to assay their use for the transformation of PAHs extracted from polluted soils
- to evidence the interference of contaminants co-extracted from the polluted soils with PAH transformation

Properties of the fungal laccases

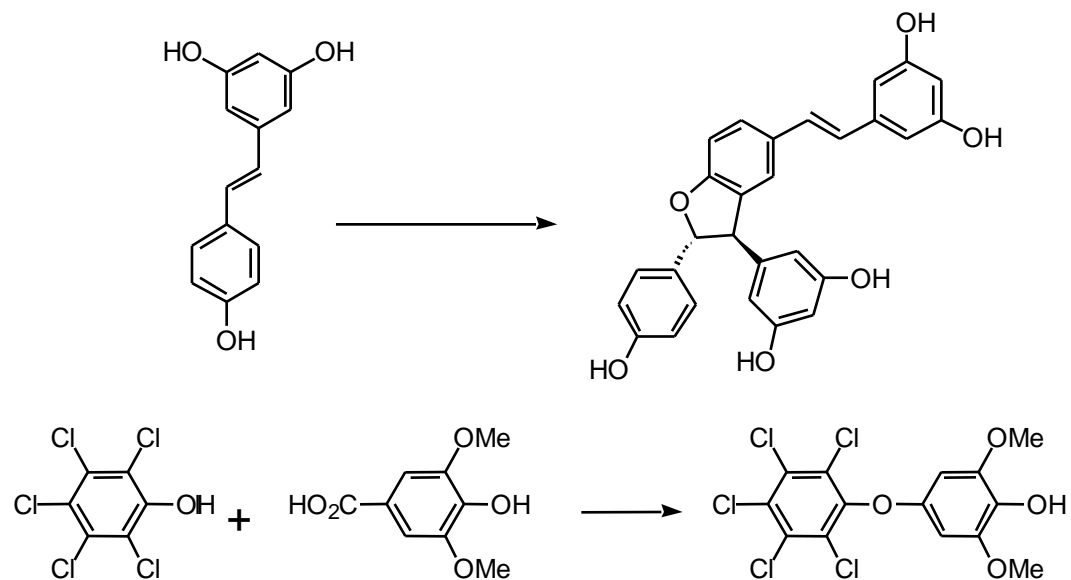
- Distribution: fungi, bacteria, higher plants, insects
- Function: lignin degradation, morphogenesis, pathogenesis
transformation of xenobiotics
- Structure: dimeric or tetrameric glycoprotein, with 4 Cu,
exocellular, 520 to 550 aa, 60 to 80 kDa
secreting a N-terminal peptide
- Regulation:
inhibitors: small anions, metals, fatty acids, chelating agents
inducers: culture medium, lignin and related compounds,
xenobiotics
- Equation: $2RH + \frac{1}{2}O_2 \rightarrow 2R\cdot + H_2O$

Main catalyzed reactions

- RH = Phenolic compound
oxidation, bond cleavage: C-C, C-N,...

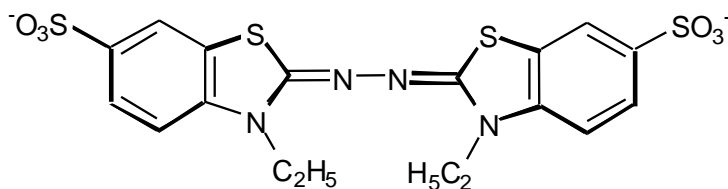
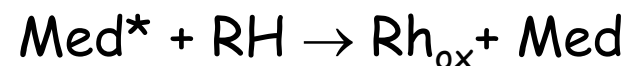


oxidative coupling

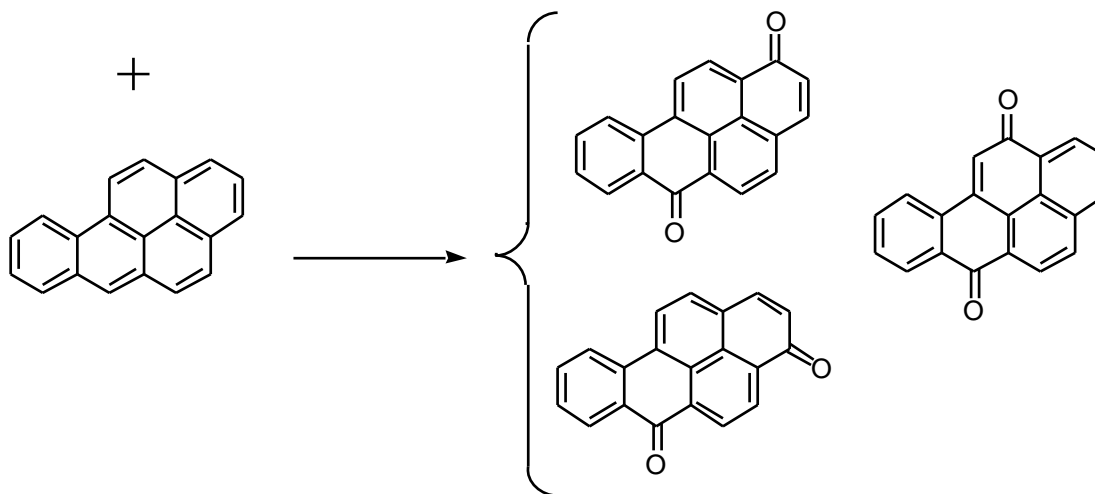


- RH = non phenolic compound

→ a redox mediator is needed: $\text{Lac} + \text{Med} \rightarrow \text{Med}^*$



2,2'-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid): ABTS



Experimental

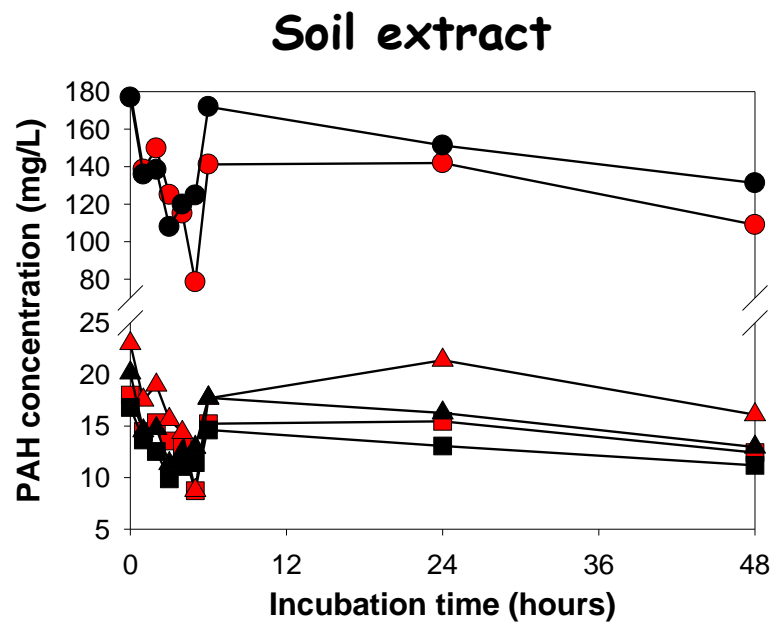
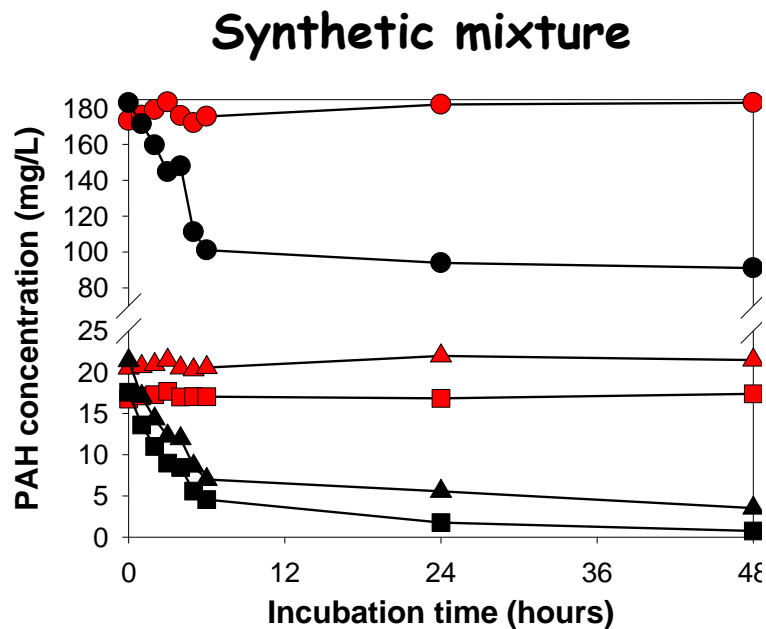


2-liter bioreactors containing:

- 1.5 L citrate/phosphate buffer pH 3, 0.1 M
- 20 mL Tween 20
- 360 mg PAHs dissolved in 500 mL acetonitrile
- 2 mM ABTS (redox mediator)
- 4000 U laccase of *Pycnoporus cinnabarinus*

incubated in the dark at 30°C for 48 hours

Transformation of PAHs

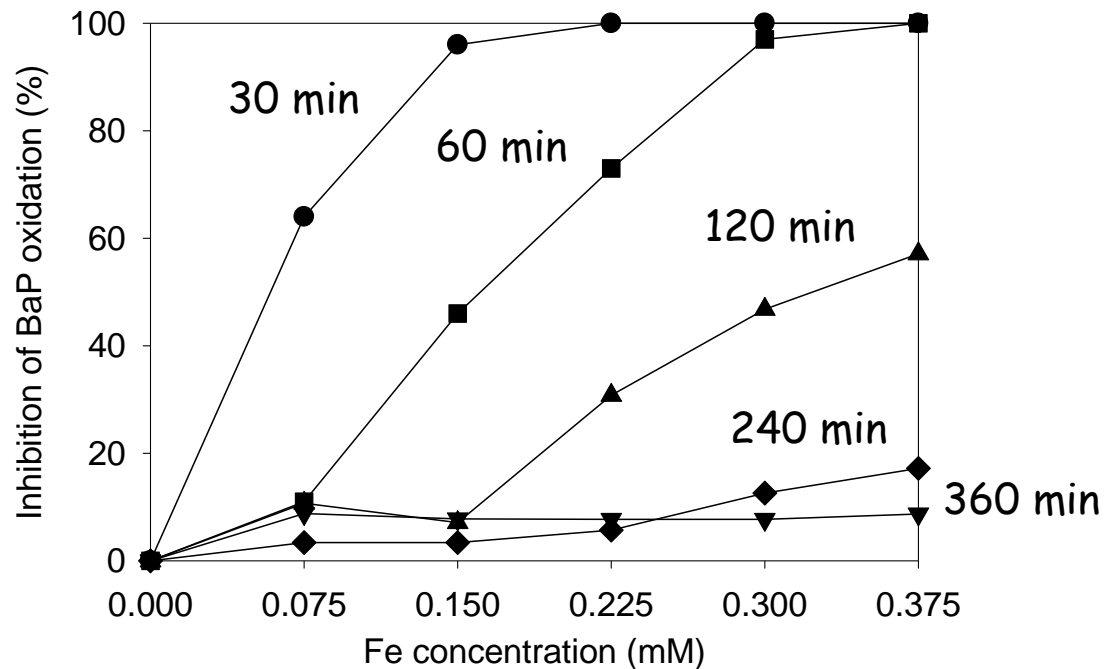


● mixture, ■ anthracene, ▲ benzo[a]pyrene, -lac, +lac

Common names	Amounts		Transformation products
	Initial (mg L ⁻¹)	After 48 h (%)	
Naphtalene	10.0	1.2	
Acenaphthylene	4.0	31.3	1,8-naphthalic acid anhydride
Acenaphtene	3.0	17.4	1,8-naphthalic acid anhydride
Fluorene	5.0	53.7	9-fluorenone
Phenanthrene	25.0	57.2	9,10-phenanthrene dione
Anthracene	17.0	4.3	9,10-anthracene dione
Fluoranthene	22.0	59.3	
Pyrene	20.0	57.3	1,6- 1,8-pyrene diones
Benzo[a]anthracene	9.0	68.6	7,12-benzo[a]anthracene dione
Chrysene	5.0	95.6	
Benzo[b]fluoranthene	17.0	63.5	
Benzo[k]fluoranthene	15.0	99.3	
Benzo[a]pyrene	21.0	16.5	1,6- 3,6- 6,12-benzo[a]pyrene diones
Dibenzo[ah]anthracene	1.0	73.7	
Benzo[ghi]perylene	1.0	80.0	
Indeno[123cd]pyrene	3.0	71.8	
16 PAHs	178.0	49.7	

Effect of $K_4Fe(CN)_6$ on BaP oxidation by $ABTS^{++}$

2 mL 0.1 M citrate-phosphate buffer pH 3: 5 mM peroxodisulfate
0.1 mM ABTS, 0.1 mM BaP, 0.075 to 0.375 mM $K_4Fe(CN)_6$



Conclusions

- fungal laccases are able to transform 10 PAHs in a synthetic mixture of 16 compounds. The reaction needs the presence of a redox mediator
 - metals, present in polluted soils, inhibit PAH transformation in withdrawing the electrons delivered by the mediator
 - as the inhibition by metals is limited in time, other unknown mechanisms may explain the lack of activity of the laccases
- Are laccases really efficient in polluted soils?
How can we enhance their potential?

Perspectives

- the results presented here have been obtained by using purified laccases from *P. cinnabarinus*. Similar data have been obtained later with enzymes from *T. versicolor*
 - we have now cloned and sequenced a gene encoding for a laccase of *T. versicolor*
 - we have expressed it in *E. coli*, soon in the yeast *Yarrowia lipolytica*
- the directed evolution of the enzyme should also be performed